MUNI MED

Ústav histologie a embryologie

"Achondroplasia Gladiator"—
Bibliotheque Nationale, Paris

Introduction to teratology

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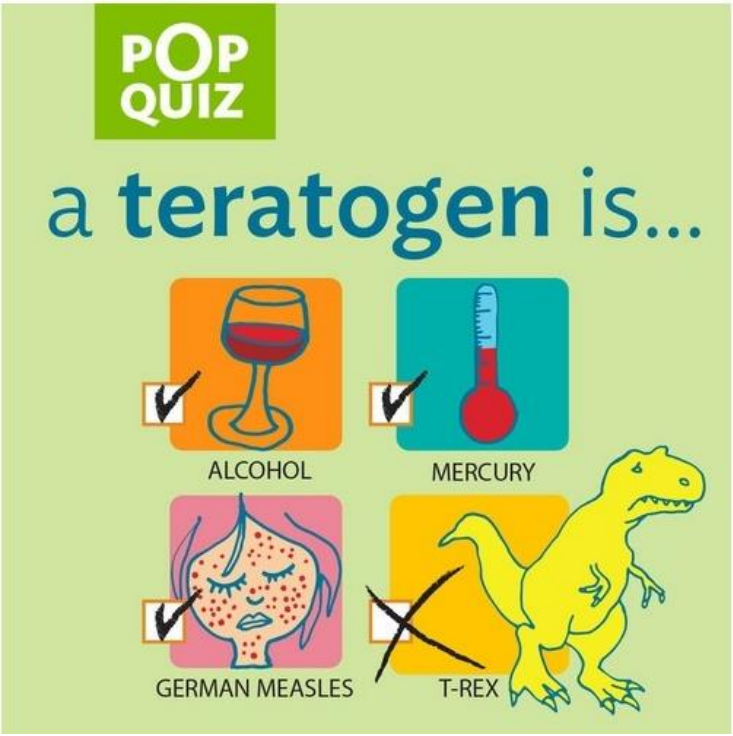
INTRODUCTION TO TERATOLOGY

Embryology is the study of normal intrauterine embryonic and fetal development.

Teratology is the study of birth defects, and a **teratogen** is something that either induces or amplifies abnormal embryonic or fetal development and causes birth defects.



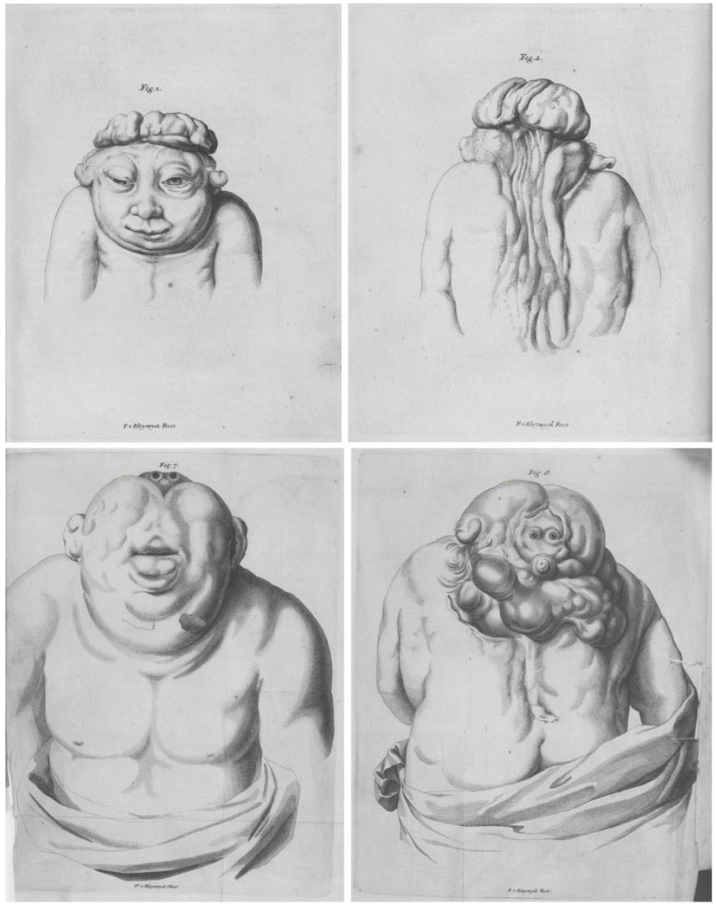
Historical context

τέρας (Greek) *teras* **= monster**

Everything looking abnormal

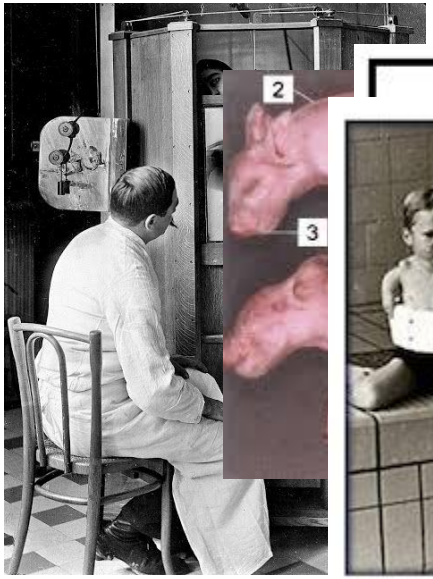
Historical context

Since the 17th century related to abnormal births (development)



- **W. Harvey** (1578–1657) - used the term "developmental arrest",
- **C.F. Wolff** (1733–1794) - in his study on the intestine, the term "germ layer" was coined that has been in use to this day,
- **A. von Haller** (1708–1777) - was first to describe the development of the chicken heart,
- **I.G. de Saint-Hillaire** (1805–1861) - was first to introduce the term "teratology",
- **C. Dareste** (1822–1899) - discussed the modes of artificial induction of monstrosities (particularly by mechanical impulses during icubation of hen eggs),
- **R. Virchow** (1821–1902) - gathered a unique collection of rare developmental disorders of the human body in the "Museum of Pathology" in the Berliner hospital Charité,
- **E. Schwalbe** (1906–1999) - defined the expression "teratogenic termination point",
- **CH.R. Stockard** (1879–1936) - introduced the term "critical period".

INTRODUCTION TO TERATOLOGY



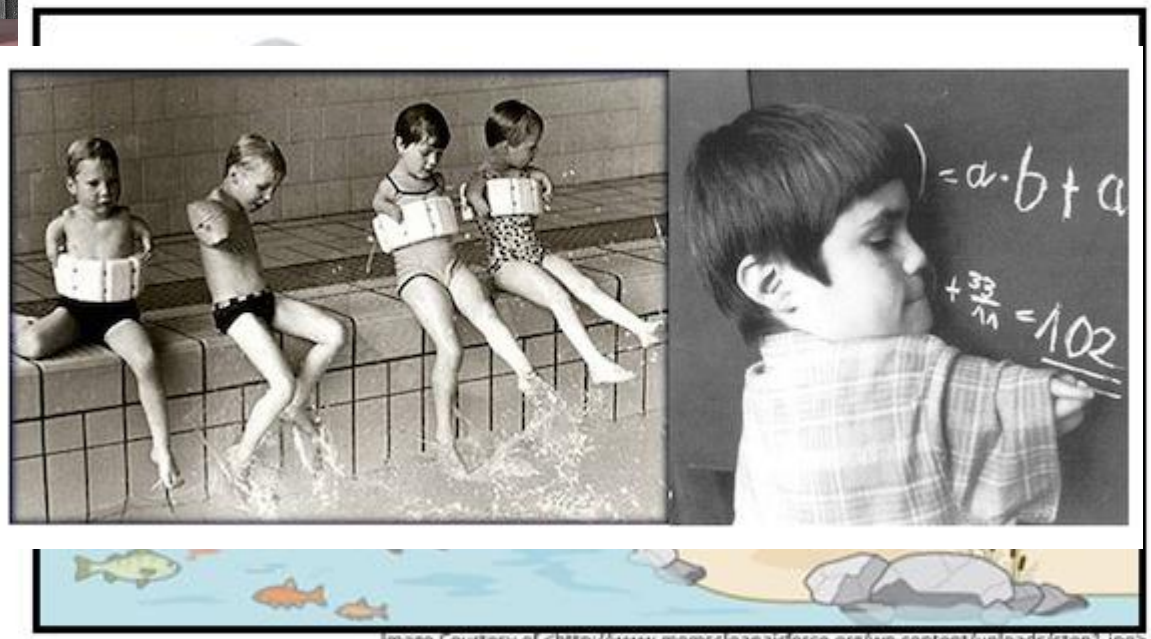


Image Courtesy of <http://www.momscleanairforce.org/wp-content/uploads/step1.jpe>

1905

1921

1929

193

1941

194

1948

1952

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1961

The first

Disorder

Embryonic lethality

The first descriptio

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The first report on human malformations induced by environ-

mental pollutants. Disc

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Thalidomide-induced embryopathy

What is a teratogen?

- X-Rays
- Lipid diet
- Hypovitaminosis
- Sex hormones
- Virus infection
- Chemical substances
- Medications
- Environmental pollutant (methyl mercury)

Teratogens around us

Physical

- Ionizing irraditation (UV, RTG, α, β, γ),
- Temperature
- Mechanical factors in utero (pes equinus, amnion bands)

Chemical

- Nucleotide analogues (chemotherapy)
- Organometall compounds
- Hormones and endocrine disruptors
- Medications (aminopterins, thalidomide, antiepileptic drugs, anticoagulants, vitamin A)
- Environmental pollutant (methylmercury, lead, heavy metals)
- Alcohol, drugs, solvents
- Many others

Biological

- Infection agents (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes, Syphilis)
- Disease of mother (diabetes, thyroid conditions, autoimmune disorders, PKU)

What does a teratogen do?

- Disrupts fine molecular or metabolic pathways

- **Intra uterine growth retardation**
- **Failure of histogenesis, organogenesis**
- **Embryonic/fetal death**

INTRODUCTION TO TERATOLOGY

Teratogen	Vrozená vada
Infekce	
rubeola virus	katarakta, glaukom, srdeční vady, hluchota, abnormality zubů
cytomegalovirus	microcephalia, slepota, mentální retardace, odumření fetu
virus Herpes simplex	microphthalmia, microcephalia, retinální dysplasie
virus varicelly	hypoplasie končetin, mentální retardace, svalové atrofie
HIV	microcephalia, růstová retardace
Toxoplasma gondii	hydrocephalia, mozkové kalcifikace, microphthalmia
Treponema pallidum	mentální retardace, hluchota
Fysikální činitelé	
rtg záření	microcephalia, spina bifida, rozštěp patra, defekty končetin
hypertermie	anencephalia, spina bifida, mentální retardace, defekty obličeje, srdeční malformace, omphalokéla, defekty končetin
Chemické látky	
thalidomid	defekty končetin, srdeční vady, hluchota, slepota, malformace dalších vnitřních orgánů
aminopterin	anencephalia, hydrocephalia, rozštěp rtu a patra
fenytoin	fetální hydantoinový syndrom, defekty obličeje, mentální retardace
kyselina valproová	defekty nervové trubice, kraniofaciální, srdeční a končetinové vady
trimethadion	rozštěp patra, srdeční, urogenitální a kosterní vady
lithium	srdeční malformace
amfetaminy	rozštěp rtu a patra, srdeční malformace
warfarin	chondrodysplasie, microcephalia
ACE inhibitory*	růstová retardace, odumření fetu
kokain	růstová retardace, microcephalia, abnormality chování, gastroschisis
ethanol	fetální alkoholový syndrom, krátké oční štěrbiny, hypoplasie maxily, srdeční malformace, mentální retardace
isotretinoin (analog vitaminu A)	embryopatie vyvolaná vitaminem A: malé abnormálně tvarované uši, mandibulární hypoplasie, rozštěp patra, srdeční vady, končetinové vady
průmyslová rozpouštědla	nízká porodní hmotnost, kraniofaciální defekty, defekty nervové trubice
organické sloučeniny rtuti	neurologické poruchy připomínající mozkovou obrnu
olovo	růstová retardace, neurologické poruchy

Teratogen	Vrozená vada
Hormony	
androgeny (ethisteron, norethisteron)	maskulinisace ženského zevního genitálu: splynulá labia, hypertrofický klitoris
diethylstilbestrol	hypoplasie varlat, malformace dělohy, vejcovodů a horní části vaginy, v dospělosti karcinom pochvy
diabetes mellitus	řada malformací, hlavně srdečních, defekty nervové trubice, syndrom kaudální regrese spojený s hypoplasií dolních končetin
obesita	srdeční vady, omfalokéla

* ACE – angiotensin-konvertující enzym

- **Growth retardation**
- **Failure of histogenesis, organogenesis**
- **Embryonic/fetal death**

How to classify a teratogen?

FDA U.S. FOOD & DRUG ADMINISTRATION

Category A: Controlled studies in women fail to demonstrate a risk to the fetus in the first trimester (and there is no evidence of a risk in later trimesters), and the possibility of fetal harm appears remote.

Category B: Either animal-reproduction studies have not demonstrated a fetal risk but there are no controlled studies in pregnant women or animal-reproduction studies have shown an adverse effect (other than a decrease in fertility) that was not confirmed in controlled studies in women in the first trimester (and there is no evidence of a risk in later trimesters).

Category C: Either studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal, or other) and there are no controlled studies in women or studies in women and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the fetus.

Category D: There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (e.g., if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective).

Category X: Studies in animals or human beings have demonstrated fetal abnormalities, or there is evidence of fetal risk based on human experience, or both, and the risk of the use of the drug in pregnant women clearly outweighs any possible benefit. The drug is contraindicated in women who are or may become pregnant.

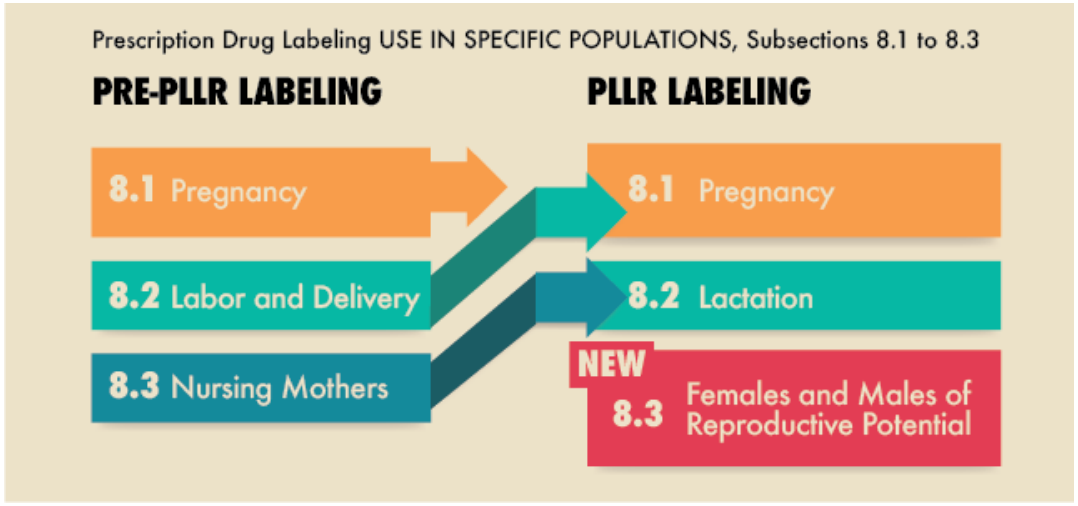
https://www.govinfo.gov/content/pkg/FR-2008-05-29/pdf/E8-11806.pdf

How to classify a teratogen?

Pregnancy and Lactation Labeling Rule (PLLR) since 2015 in USA

Instead of using an arbitrary lettering system, the PLLR provides detailed risk summaries and more comprehensive information derived from clinical experience (if available), animal data, and concerns related to the pharmacologic activity of the drug.

In addition, the label includes information on the risks associated with untreated illness. This information helps to put the potential effects of the drug into perspective with the goal of providing a more individualized risk-benefit analysis.



FDA U.S. FOOD & DRUG ADMINISTRATION

https://eu-rd-platform.jrc.ec.europa.eu/eurocat/prevention-and-risk-factors/medication-during-pregnancy_en

How to avoid teratogen?

- Talk with your healthcare provider about any medications you're taking.
- Avoid cigarettes, alcohol and recreational drugs.
- Don't take any supplements, medications or prescription drugs without checking with your healthcare provider.
- Avoid cleaning litter boxes.
- Avoid hot tubs, saunas and anything that raises your internal body temperature.
- Remove tuna, swordfish and other fish high in mercury from your diet.
- Talk with your supervisor or human resources about harmful chemicals in your workplace.

Cleveland Clinic

How to identify a teratogen?

<5%

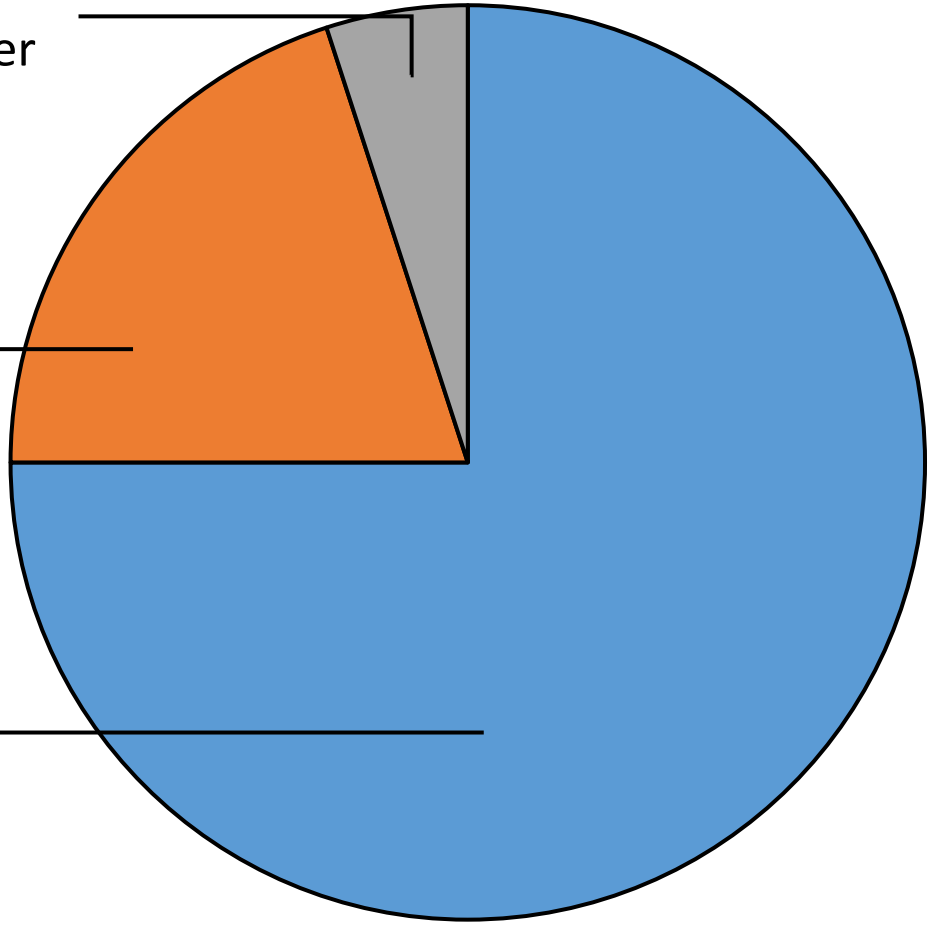
- Environmental
- Intrauterine infections
- Metabolic disorder of mother
- Drugs and medications
- Radiation

20-25%

Genetical

65-75%

Multifactorial or unknown



How to identify a teratogen?

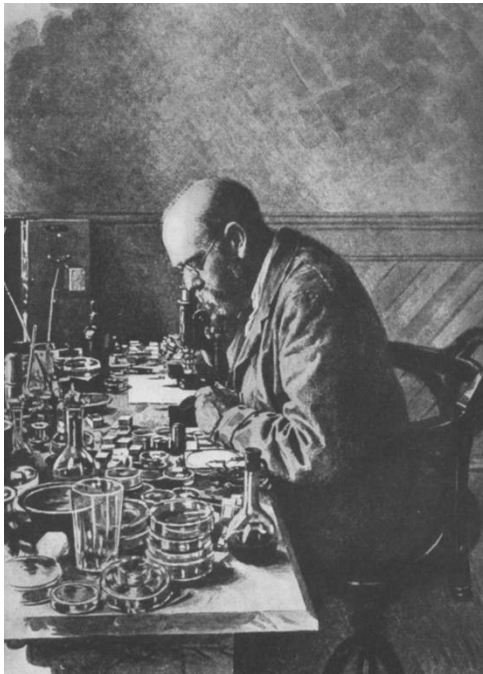
- **Animal studies**
- **Observations from human exposure**

Koch's Postulates in microbiology:

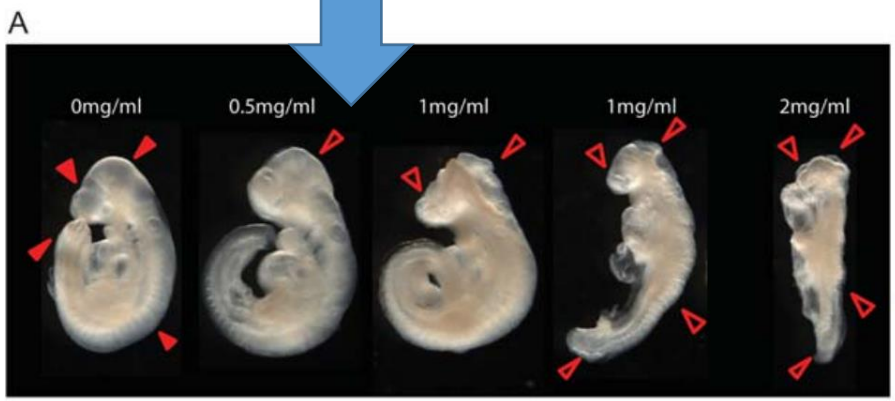
- *The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.*
- *The microorganism must be isolated from a diseased organism and grown in pure culture.*
- *The cultured microorganism should cause disease when introduced into a healthy organism.*
- *The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.*

"Koch's Postulates„ adapted for teratology:

- The agent must be present during the critical stage of development.
- The agent produces a particular pattern of birth defects in animal studies.
- The agent crosses the placenta and there is a dose-response relationship.
- There is an abrupt increase in the frequency of a particular defect or group of defects (syndrome).
- The increase of defects is associated with the use of a new drug or the widespread exposure to a chemical or environmental change.
- There is an absence of other factors to explain the observations.
- The mechanism of teratogenesis makes biological sense.



INTRODUCTION TO TERATOLOGY



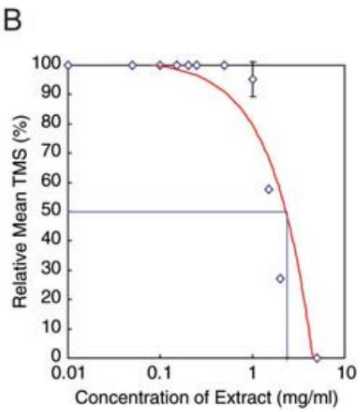


Figure 1 Embryotoxicity of LAR in mouse embryo culture. (**A**) Abnormal embryonic development and anomalies were observed in treatment groups from 0.5 to 2 mg/ml of LAR. Representative embryos are presented. Closed arrows represent the normal structures. Open arrows represent the malformed structures, including microencephaly, opened neural tube defects and sacral regression. Magnification ×25. (**B**) Dose–response curve of LAR on embryo development. The minimum concentration of LAR which resulted in a 50% reduction in relative TMS in the embryos was indicated. $n = 10$ embryos per each LAR concentration.

Molecular Human Reproduction, Vol.18, No.12 pp. 585–592, 2012
Advanced Access publication on August 19, 2012 doi:10.1093/molehr/gas034

MHR ORIGINAL RESEARCH

Molecular studies of the congenital malformation induced by *Largehead Atractylodes Rhizome*, the most commonly used Chinese medicine for threatened miscarriage

L.Y. Tang[1,†], L. Li[1,†], A. Borchert[2], Clara B.S. Lau[3], P.C. Leung[3], and C.C. Wang[1,4,*]

[1]Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong [2]Institute of Biochemistry, University of Medicine Berlin, Charité, Berlin, Germany [3]Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong [4]School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

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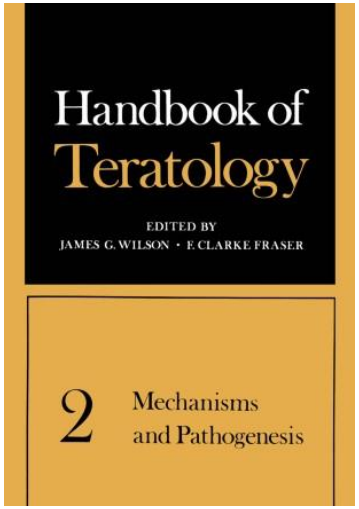
Submitted on March 27, 2012; resubmitted on August 3, 2012; accepted on August 9, 2012

How to identify a teratogen?

- Animal studies
- Observations from human exposure

- **Understand the biological context**

INTRODUCTION TO TERATOLOGY



Wilson's Six Principles of Teratology (1977)

1. Susceptibility to teratogenesis depends on the **genotype** of the conceptus and the way in which this interacts with environmental factors.

2. Susceptibility to teratogenic agents varies with the **developmental stage** at the time of exposure.

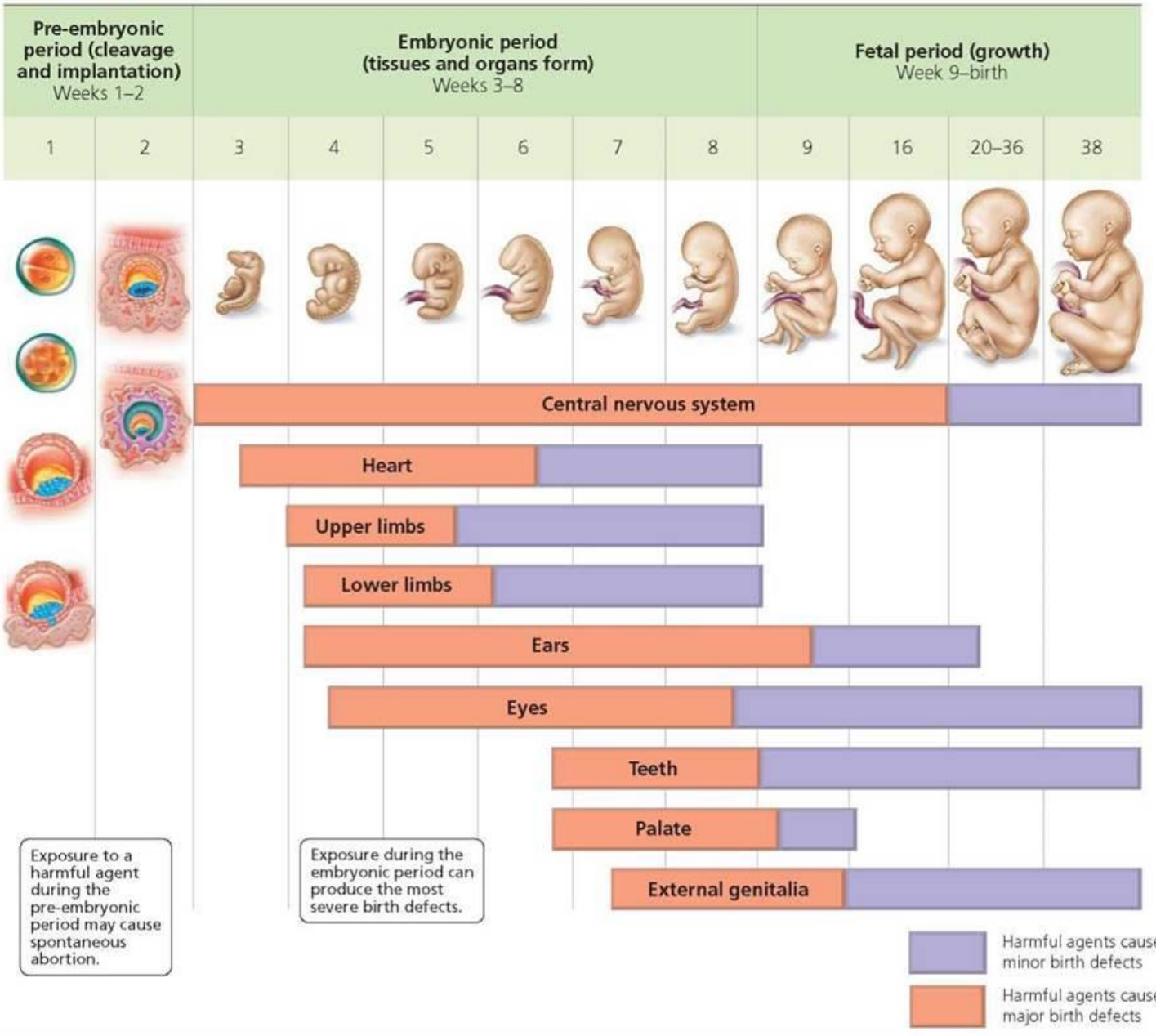
3. Teratogenic agents act in **specific ways** (mechanisms) on developing cells and tissues to initiate abnormal embryogenesis (pathogenesis).

4. The **final manifestations** of abnormal development are death, malformation, growth retardation, and functional disorder.

5. The access of adverse environmental influences to developing tissues depends on the **nature of the influences** (agent).

6. Manifestations of deviant development increase in degree as **dosage** increases from the no-effect to the totally lethal level.

INTRODUCTION TO TERATOLOGY



Mechanisms of action?

- Mutation
- Chromosomal nondisjunction and breaks Mitotic interference
- Altered nucleic acid integrity or function
- Lack of precursors and substrates needed for biosynthesis
- Altered energy sources
- Enzyme inhibitions
- Osmolar imbalance
- Altered membrane characteristics

1977

- Epigenetic control of gene expression
- The effects of small regulatory RNAs
- The imbalance of gene products resulting from submicroscopic alterations of genomic structure such as copy number changes
- Alterations of the cytoskeleton
- Perturbations of the extracellular matrix
- Effects of mechanical forces on embryogenesis
- Disturbances of intracellular or intercellular signalling
- Dysfunction of molecular chaperones
- Effects on the distribution of molecules into subcellular compartments
- Alterations of the integrity of intracellular organelles

2010


ZIIKA FOREST
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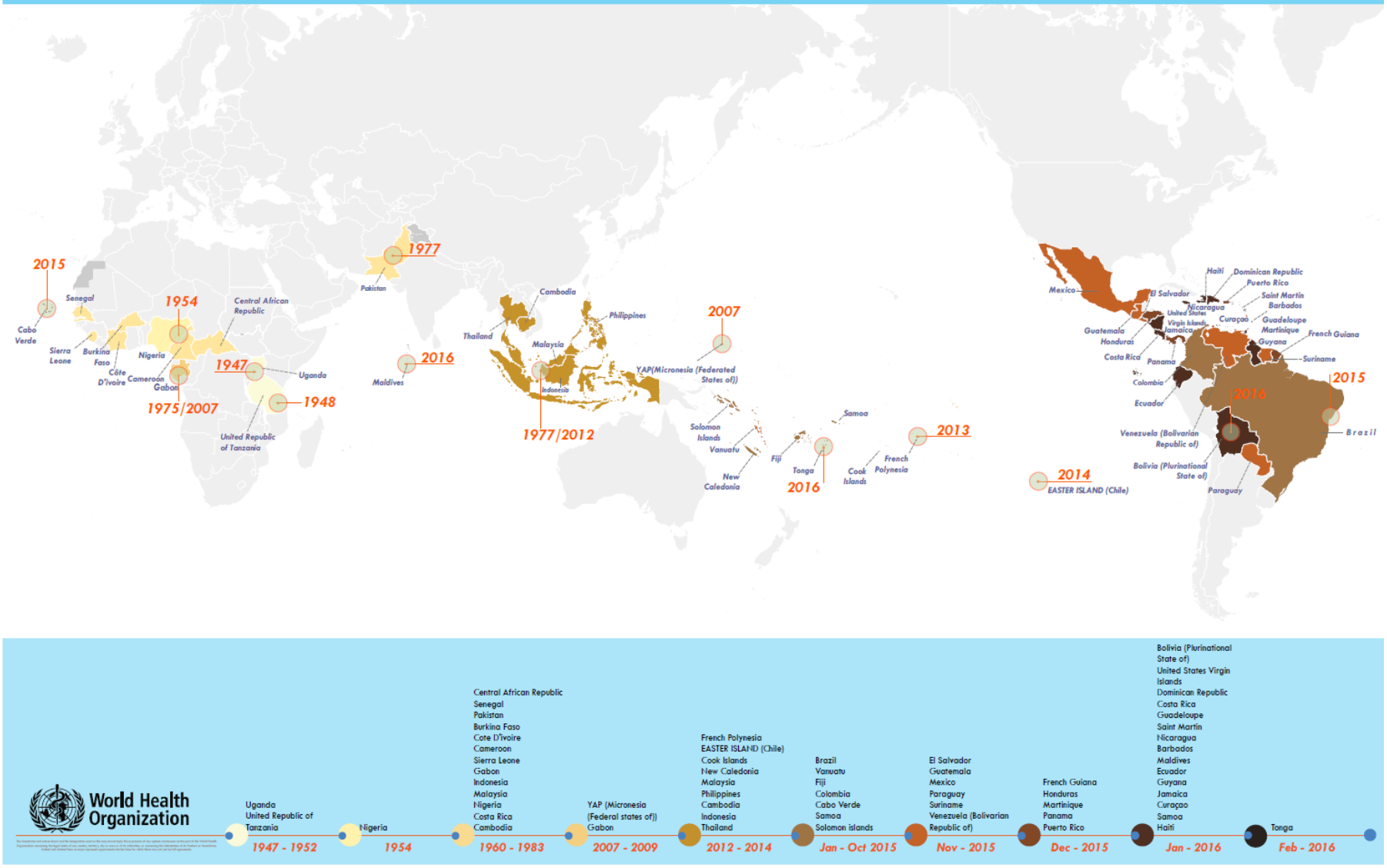
CASE STUDY

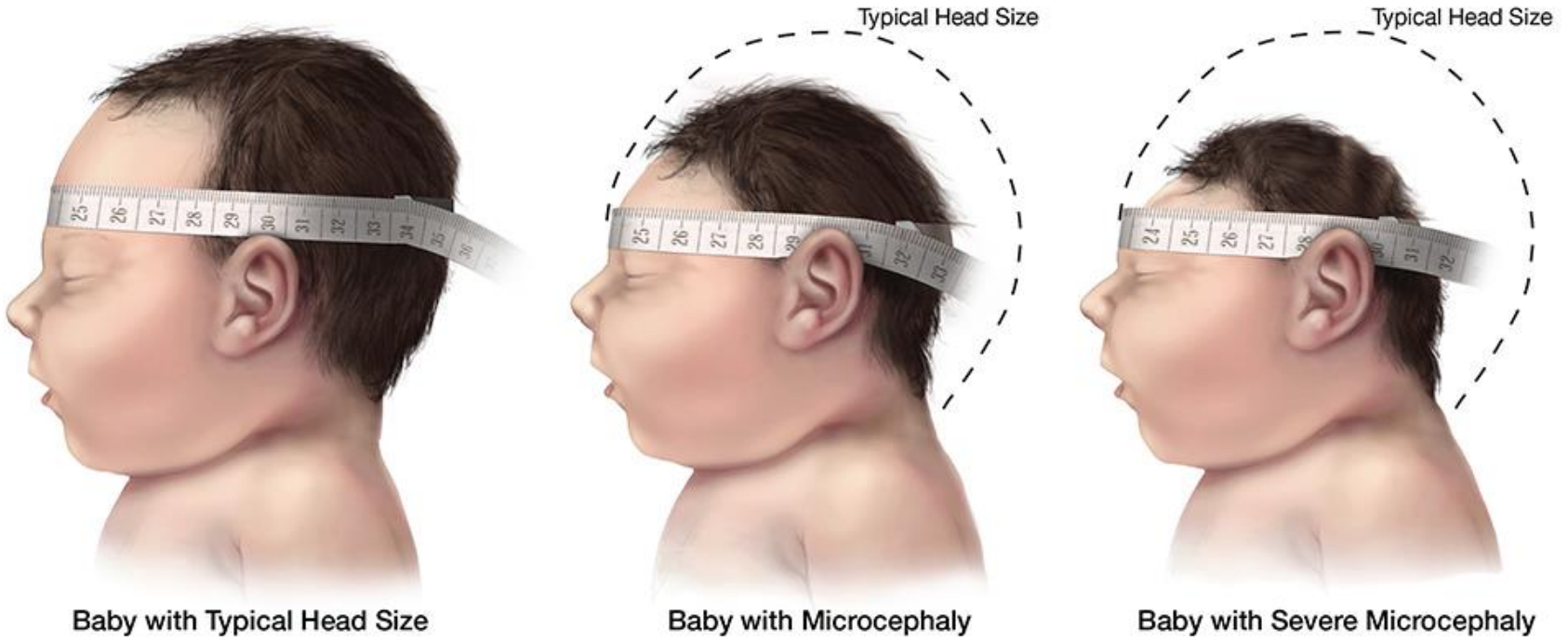
Countries and territories showing historical time-line of Zika virus spread (1947 - 2016)



World Health Organization

Uganda United Republic of Tanzania	Nigeria	Central African Republic Senegal Pakistan Burkina Faso Cote D'Ivoire Cameroon Sierra Leone Gabon Indonesia Malaysia Nigeria Costa Rica Cambodia	YAP (Micronesia (Federal states of)) Gabon	French Polynesia EASTER ISLAND (Chile) Cook Islands New Caledonia Malaysia Philippines Cambodia Indonesia Thailand	Brazil Vanuatu Fiji Colombia Cabo Verde Samoa Solomon Islands	El Salvador Guatemala Mexico Paraguay Suriname Venezuela (Bolivarian Republic of)	French Guiana Honduras Martinique Panama Puerto Rico	Bolivia (Plurinational State of) United States Virgin Islands Dominican Republic Costa Rica Guadeloupe Saint Martin Nicaragua Barbados Maldives Ecuador Guyana Jamaica Curaçao Samoa Haiti	Tonga
1947 - 1952	1954	1960 - 1983	2007 - 2009	2012 - 2014	Jan - Oct 2015	Nov - 2015	Dec - 2015	Jan - 2016	Feb - 2016

Evidence for ZIKV induced microcephaly?



How to identify a new teratogen?

- Observations from human exposure
- Understand the biological context
- Validate on animals

Bradford Hill criteria

Formerly published as Medico-Chirurgical Transactions • 1809 – 1907
Proceedings of the Royal Society of Medicine
Now published as Journal of the Royal Society of Medicine • 1978 – current

Proc R Soc Med. 1965 May; 58(5): 295–300. PMCID: PMC1898525
PMID: 14283879

The Environment and Disease: Association or Causation?

Austin Bradford Hill

Strength (effect size): A small association does not mean that there is not a causal effect, though the larger the association, the more likely that it is causal.

Consistency (reproducibility): Consistent findings observed by different persons in different places with different samples strengthens the likelihood of an effect.

Specificity: Causation is likely if there is a very specific population at a specific site and disease with no other likely explanation. The more specific an association between a factor and an effect is, the bigger the probability of a causal relationship.

Temporality: The effect has to occur after the cause (and if there is an expected delay between the cause and expected effect, then the effect must occur after that delay).

Biological gradient: Greater exposure should generally lead to greater incidence of the effect. However, in some cases, the mere presence of the factor can trigger the effect. In other cases, an inverse proportion is observed: greater exposure leads to lower incidence.

Plausibility: A plausible mechanism between cause and effect is helpful (but Hill noted that knowledge of the mechanism is limited by current knowledge).

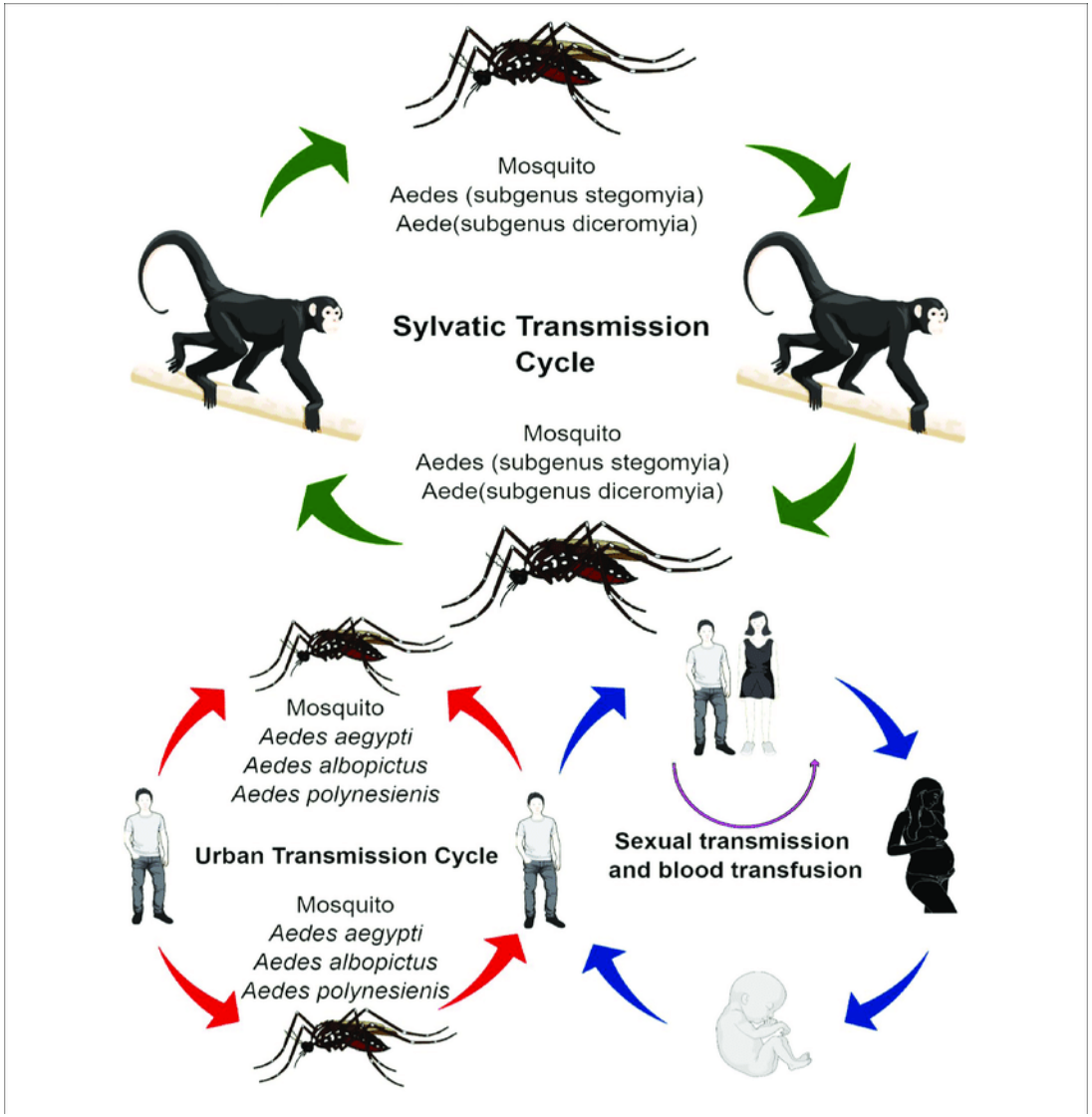
Coherence: Coherence between epidemiological and laboratory findings increases the likelihood of an effect.

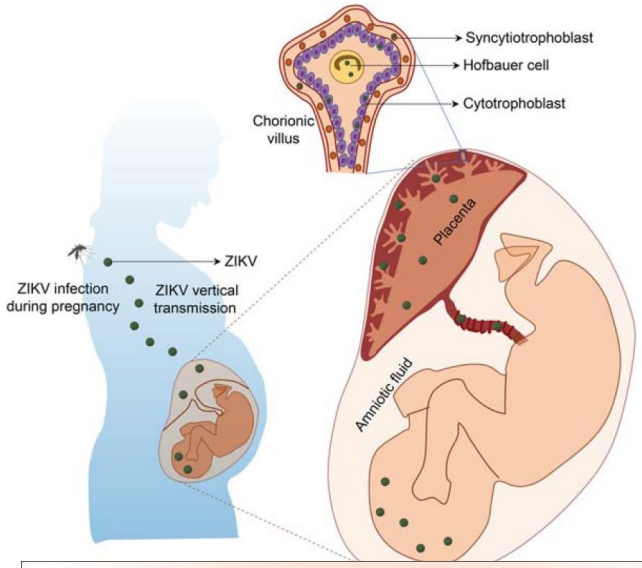
Experiment: "Occasionally it is possible to appeal to experimental evidence".

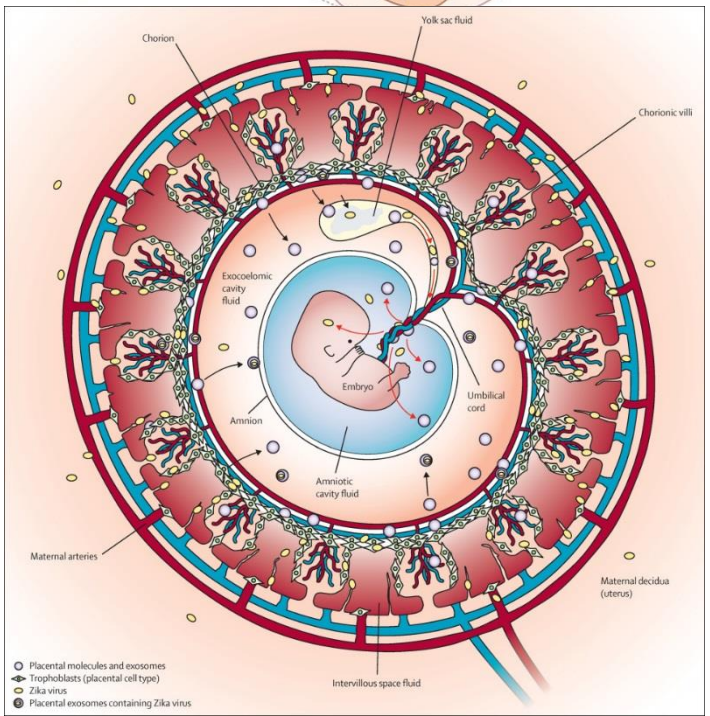
Analogy: The use of analogies or similarities between the observed association and any other associations.

Reversibility: If the cause is deleted then the effect should disappear as well.

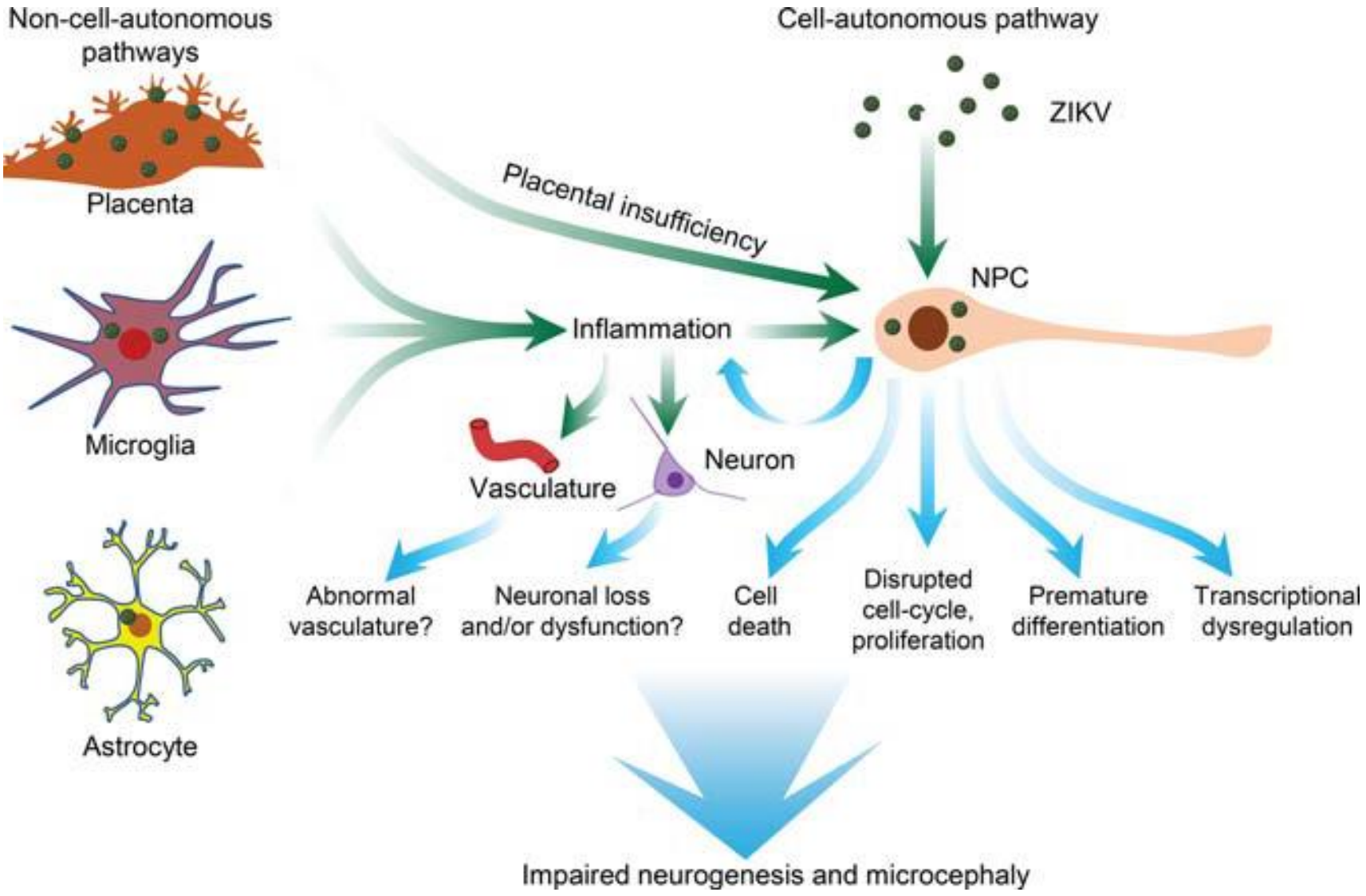
ZKV transmission







ZKV mechanism of action



Take home message and further reading

- Teratology, teratogens → From Koch's postulates to Wilson's principles
- Mechanisms of action → any embryology and/or cell biology textbook
- Classification & clinical examples → any embryology textbook, FDA (EU) categories
- Identification, validation → ZIIKA forest virus story & Bradford Hill criteria

Suggested reading:

- Friedman JM. The Principles of Teratology: Are They Still True? Birth Defects Research (Part A): Clinical and Molecular Teratology 88:766–768 (2010)
- Varga I. Embryology Teaching: An Often-neglected Part of the Medical Curriculum. Rev Arg de Anat Clin. 9(2):47-51 (2017)
- Ujhazy et al. Teratology – past, present and future. Interdiscip Toxicol. 5(4): 163–168 (2012)

Not every genetic anomaly results in a malfunction

X

Thank you for attention

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RESEARCH

Leaf Trait Coloration in White Clover and Molecular Mapping of the Red Midrib and Leaflet Number Traits

Rebecca M. Tashiro, Yuanhong Han, María J. Monteros, Joseph H. Bouton, and Wayne A. Parrott*